Lab Session 18-08 to 24-08

13 September 2015 22:45

Lab Session 18.08.2015

1. Digestion of NrfA, CysI and Nosz with XbaI :

- Reduction volume: 20 uL or 50 uL.
- Plasmids of these parts : 300 ng/uL
- Digested with XbaI
- 1 uL on gel > to verify

1. Filling reaction to do blunt end ligation.

- We have linearised PSB1C3
- Fill reaction with vent polymerase.

1. Purification of the Single digested product:

- Purified and eluted in 30 uL.
- 50 ng/uL NosZ and CysI.
- 30 ng/uL.

1. Digestion of purified production with SpeI.

- Gel Elute of 415 R.P = 1440 bp
- 50 uL reaction
- DNA > 30 uL
- Enzyme > 0.5 uL
- Buffer > 5 uL (10 times off enzyme)
- Water > 14.5 uL
- Analytical gel.

1. 2:1 Ligation.

- Equimolarity
- Smallness of Influx.
- 277K overnight.
- Blunt ends didn't ligate well at room temperature.

Other than that, PCR for primer was checked.

- 3 uL NosZ Duplicate
- 3 uL NosZ 0.5 uL primer
- 1.5 uL NosZ
- Control: no NosZ

Lab Session 19.08.2015

The following things were done:

- Again digestion of NrfA, CysI and NosZ were done, this time with XbaI.
  - Reaction Volume: 20 uL and 7 uL of plasmid. (Conc. : 2000 ng/uL).
- Inoculation of NrfA, CysI, NosZ in 20 mL falcon.
- Chloramphenicol stocks were made.
  - Stock: 34 mg/ml in 1 mL.
  - Digestion was performed, followed by filling in reaction for 30 min.
- J23119 + NrfA (from last year) was found to be a success. It was confirmed by sequences.
- We also found sequences of J23119.
- Consequently, inoculation was set up for the J23119 stocks.
- We were basically aiming for the RBS to be revived.

Lab Session 20.08.2015

Pbcks (+) strategy:

1. Digestion with XbaI
2. Filling Reaction
3. Purification done.
   - NrfA > 55.9
   - NosZ > 98.9
   - CysI > 70.9
4. Digestion with SpeI
   - NrfA > 23
   - NosZ > 23
   - CysI > 21
5. Aliquot run to verify
6. Gel Run
7. Ligation overnight.

Ligation:

- NrfA, NosZ, CysI:
  - Compatible with EcoRI and SpeI purified.
  - Ready for Digestion > 22 ng/uL

- Pbcks(+)
  - Digested until EcoRI and SpeI.
  - Purification was done.
  - Concentrations were checked.
  - Finally, they were ready for ligation.
- Ligation was set up.

Making pr+RBS:

1. Electroporation of RBS
2. Plating
3. Colonies were obtained
4. Inoculation in LB.

Lab Session 21.08.2015

Transformations were done, following a different procedure:

- 1.5 mL of culture was set up in each tube and centrifuged for 10 minutes.
- Resuspension was done in 1 mL of chilled water.
- The cells were centrifuged for 10 min at 10,000 rpm.
- The supernatant was discarded.
- 1 uL of DNA was put into the samples:
  - B0034 > 5.6 sec
  - B0030 > Arc + 4.3 sec > used 0.6 uL
  - KS92024 > 5.4 sec.
  - K864101 > 5.6 sec.
- 1 uL of media was prepared.
- Fresh Eppendorf's tubes of 2 ml were taken and labelled.
- The cells were put, and electric shocks were provided.
- LB was put right way to create isotonic solution.
- It was put in the incubator for 30 mins. (Temp. > 310 K.)
- Pellets settle down.

Digestion Set up:

- NrfA (47 uL) > 169.7. Digestion with XbaI.
  - Enzyme : 1 uL
  - Buffer : 5 uL
  - Plasmid : 44 uL
  - Time: For 210 mins.
- CysI (44 uL) > 184.9. Digestion with XbaI.
  - Enzyme : 1 uL
  - Buffer : 5 uL
  - Plasmid : 44 uL
  - Time: For 210 mins.

- NosZ (49 uL) > 185.6. Digestion with XbaI.
  - Enzyme : 1 uL
  - Buffer : 5 uL
  - Plasmid : 39 uL
  - Water : 6 uL
- Pbcks(+). Digestion with EcoRV:
  - Plasmid : 3 uL
  - Buffer: 1 uL
  - Enzyme: 0.5 uL
  - Water : 5.5 uL
- Pbcks(+). Digestion with SpeI:
  - Plasmid : 3 uL
  - Buffer: 1 uL
  - Enzyme: 1 uL
  - Water : 5 uL

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Digestion Reaction:

EcoRV Pbcks(+) SpeI (3.4 kp)

EcoRV SpeI (25 bp) (2)

XbaI Psb1c3 SpeI (2.0 kb) (3)

XbaI Psb1c3 Spe1 (1.4/1.6/1.9 kb) (4)

In Psb1c3 :

	NrfA	CysI	NosZ

BamII-SacI	No	No	No
PvuII	No	Yes	Yes
BsaHII	Yes	No	No
ZraI	No	No	Yes
AaatII	No	No	Yes

Ligation was performed then. For that,

Insert: 22 ng/uL (Conc.)
Vector: 9 ng/uL (Conc.)

- SET I:

Ratio > Insert : Vector = 4 : 1

(uL : Volume)	NrfA	NosZ	CysI
Buffer	2	2	2
Enzyme	1	1	1
Vector	4.7	4.2	4.3
Insert	2.3	2.8	2.7

- SET II:

Ratio > Insert : Vector = 5 : 1

(uL : Volume)	NrfA	NosZ	CysI
Buffer	2	2	2
Enzyme	1	1	1
Vector	3.9	3.3	3.6
Insert	3.1	3.7	3.4

Lab Session 23.08.2015

- Firstly, we checked for the presence of periplasmic NosZ. It was found to be present.
- The RBS problem was solved with proper strategy.
- PSL 8 blunt end ligation was checked.

Pbcks(+) set I:

- Ligation.
- Transformation.
- Colonies till 8 pm.
- Inoculation.
- Plasmid Isolation.
- Digestion.

Lab Session 24.08.2015

Today's work:

- Inoculation was done for: K741006 13 L
- Glycerol stocks were revived for Pr + NrfA
- Cloning was done for : PelB + Pr.
- Finally, inoculation was set up for PelB.